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LONDON, W.12

7 November, 1954.

Hfr. $V_6^s \times F- = V_6^r$

Dear Joshua,

Thanks for your letter of Sept.16. I, too, have been more or less completely involved in teaching, administration and routine work of one kind or another and it is only about six weeks ago that I started research again. You said you were interested in my work on the kinetics of recombination using the T phages. At present I am rather concentrating on clearing this up for publication. I started using T3 since, in the Hfr x F- cross, the V_3^s character of the Hfr parent appeared virtually absent from prototrophs. T3, however, was not completely satisfactory since high titre preparations contained sufficient host-range mutants which periodically produced lysis of the F- parent as well. I have now gone over to T6 which, despite inheritance of V_6^s from the Hfr parent by 20-30% prototrophs, works well in practice. What I do is roughly as follows. Young, aerated parental cultures are mixed in equal proportions and aerated at 37°. At 0, 5, 15, 30, 60, 90 minutes, 1.0 ml.vols. are removed to small tubes containing 1.0 ml.vols. phage at a multiplicity of 50-100, and aerated for 15-30 minutes. The mixtures are washed x 3, diluted appropriately (usually to about 10^{-3} of the original volume) and 0.01 ml.vols. plated on MA + B_1 in 3 cm. plates. There are usually no prototrophs, or only 1-5, at 0 mins., a few at 5 mins. and then the count rises very rapidly to 100 or more. The maximum ratio of prototrophs to F- cells occurs after about 30 mins. while the total number of prototrophs does not rise much after 60 mins. I have extended the method to analysis, under rather artificial conditions, of segregation. In the system Hfr. $V_1^r.V_6^s \times F- V_1^s.V_6^r$, the parental mixture is first treated with T_1 for the minimum period giving a good yield of prototrophs - about 15 mins. After washing, the mixture is plated in replicate. At intervals thereafter, high titre washed T_1 is spread over the surface of duplicate plates of the series and, at the same time, a similar vol. of distilled water is spread over a corresponding pair of plates (i.e. a Newcombe type of experiment is performed). The T_1 effectively destroys the F- parental cells and unsegregated zygotes and the only things that can survive are segregants which have inherited the Hfr T_1^r character and have become phenotypically expressed. By this method, the phenotypic expression of segregants from zygotes formed in broth appears to take about 4 hours on MA + B_1 and this roughly corresponds to the time taken for the first division of segregants as judged by increasing counts on the "Newcombe" plates. These are the bones of the business. I am at present engaged in using these techniques as a tool in investigating the details of zygote formation and segregation at the genetic level: for example, by using T_1 instead of T6 to destroy the Hf parent and studying the inheritance of V1 and the markers linked to it

among the resulting prototrophs, T_1 having been removed, of course, before plating. I think this promises to be a profitable line and I already seem to have run into several paradoxes, especially in regard to crosses reversed for T_1 which are analysed by this technique. I will write again when I have some definite information. We are holding a colloquium on bacterial genetics in London from Dec.14-17. Stocker, Pollock, Rowley and myself are running it and the attendance will be about 100. I will be giving a resumé on recombination (Jinks & Calef will also speak). Have you anything new I can give them about your work? I hear persistent rumours about your actually observing the recombination process by means of a motility technique. Is this true? I also wonder if Luca might be in England at that time. Give him and his wife my best regards: I will write to him. Many thanks for the offer of the Het strain. Just at the moment I would'nt know what to do with it! Please send me a reprint of your paper with Nelson in the Proc.Nat.Acad,Sc.on the analysis of elimination patterns in diploids. I learn that you are having a sabbatical year and coming to Europpe. You must pay a visit to London! I still have'nt got any line on F. It's a devil. Have you succeeded in isolating the segregants from a zygote with a micromanipulator? This would seem the only effective way of analysing unselected progeny adequately, since secondary (and tertiary) recombination must certainly occur within colonies on nutrient agar, derived from a zygote which has segregated the Hfr parental type. Not having a micromanipulator, I have in mind to let the segregants swim apart on a moist agar surface before immobilising them by drying. This is a very patchy letter. Best wishes to yourself and Esther. Please let me know what you can for our colloquium. Yours, Bill

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BY AIR MAIL

PAR AVION
AIR LETTER
AEROGRAMME

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Second fold here

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